

## **REMARKS**

By the above amendment, Applicants have amended Claims 1 and 3 to correct typographical error.

### **The Objection To Claim 1 Under § 112**

The Examiner has objected to claim 1 due to the presence of the misspelled term “hormone”. Claim 1 and claim 3 have been modified to correct the typographical error.

Thus the objection would appear to be avoided.

### **Rejection under 35 USC 103(a):**

The examiner rejected claims 1-11 and 23-28 under 35 U.S.C 103 (a) as being unpatentable over the disclosures of Markussen et al. (USPN 4,962,212 hereafter ‘212) in view of Schweden et al. (USPN 5,672,487 hereafter ‘487).

Applicants respectfully traverse this rejection and, to the extent they are maintained with respect to the claims as amended herein, request reconsideration and withdrawal of the rejections.

There is no justification, in Markussen et al. and Schweden et al., or in any other prior art separate from applicants’ disclosure, which suggests that these references be combined, much less be combined in the manner proposed.

US Patent 4,916,212 discloses a DNA sequence comprising a sequence encoding an insulin precursor of formula B(1-29)-(X<sub>n</sub>-Y)<sub>m</sub>- A(1-21) wherein m= 0 or 1. There are specific disclosures of expression of B(1-29)-A(1-21) using Mf-alpha signal/leader sequences (Col. 4, ln. 40-47). The ‘212 patent describes very broad generic methods for expression of insulin precursors. The ‘212 patent does not disclose expression using *Schwanniomyces occidentalis* glucoamylase or *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequences.

The US patent 5,672,487 discloses construction of vectors for the secretory expression of recombinant protein specifically discloses for hirudin, from the yeast *Hansenula polymorpha*. The glucoamylase leader sequence from *Schwanniomyces occidentalis* glucoamylase or *Carcinus maenas* are disclosed as an applicable signal peptide sequences. The '487 patent does not discloses the use of *Schwanniomyces occidentalis* glucoamylase or *Carcinus maenas* as an signal peptide sequence for the expression of insulin in yeast. The '487 patent does not suggest, motivate to replace Mf-alpha signal peptide sequence with the signal peptide sequences from *Schwanniomyces occidentalis* glucoamylase or *Carcinus maenas*.

Furthermore the '212 patent does not teach or motivate one of ordinary skilled in the art to replace Mf-alpha signal peptide sequence with the signal peptide sequences from *Schwanniomyces occidentalis* glucoamylase or *Carcinus maenas* which would result in very high expression of the pro-insulin using the expression system disclosed in the our application. Hence the invention as claimed in our application is a significant technological advancement over the cited prior art.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

Furthermore applicants would like to draw the attention of the examiner to the fact that recombinant protein expression in foreign host is highly unpredictable. There are number of peer reviewed research findings supporting the same, a few examples are cited as under:

- 1) Laforet et al teach that signal peptide subsegments are not always functionally interchangeable (J Biol Chem. 1989 Aug 25;264(24):14478-14485).
- 2) Kjaerulff, S. & Jensen M. R., *Biochem Biophys Res Commun*. 2005, 336 : 974-82. "Comparison of different signal peptides for secretion of heterologous proteins in fission

yeast.”

Kjaerulff et al discloses “In the fission yeast *Schizosaccharomyces pombe*, there are relatively few signal peptides available and most reports of their activity have not been comparative. Using sequence information from the *S. pombe* genome database we have identified three putative signal peptides, designated Cpy, Amy and Dpp, and compared their ability to support secretion of green fluorescent protein (GFP). In the comparison we also included the two well-described secretion signals derived from the precursors of, respectively, the *Saccharomyces cerevisiae* alpha-factor and the *S. pombe* P-factor. The capability of the tested signal peptides to direct secretion of GFP varied greatly. The alpha-factor signal did not confer secretion to GFP and all the produced GFP was trapped intracellular. In contrast, the Cpy signal peptide supported efficient secretion of GFP with yields approximating 10 mg/L.”

3) Murasugi, A. & Tohma-Aiba, Y; Biosci. Biotechnol. Biochem. 2001, 65(10), 2291-3. Comparison of three signals for secretory expression of recombinant human midkine in *Pichia pastoris*.

Murasugi et al discloses “The secretion signals of *Saccharomyces cerevisiae* alpha mating factor, human midkine itself, and *Pichia pastoris* acid phosphatase, were tried for the expression of human midkine under the control of the AOX1 gene promoter in *P. pastoris*. Approximately 28 mg/l, 1.5 mg/l, and 0.2 mg/l of midkine were secreted by using the mating factor pre-pro-sequence, the midkine signal sequence, and the phosphatase signal sequence in flask cultures, respectively.”

The above studies conclude that results obtained for protein secretion utilizing different signal peptide sequences for example alfa mating factor signal from *Saccharomyces cerevisiae* peptide support high protein secretion in *Pichia* host cell system then the native phosphatase signal peptide driven secretion of the same. Thus indicating the unpredictable nature of the recombinant protein expression, in other words

expression of recombinant protein in foreign host is not guaranteed even by use of well characterized signal sequences.

As a result, it is difficult for one of ordinary skilled in the art to predict that his invention will work for all products even in same or different organisms.

Moreover *Schwanniomyces occidentalis* glucoamylase and *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequences are very different from Mf-alpha signal sequences in terms of their origin as well as sequence. While Mf-alpha signal sequences disclosed in the cited prior art '212 are derived from the yeast *Saccharomyces cerevisiae*, the signal peptide used in our application is derived from *Schwanniomyces occidentalis*, an entirely different yeast genus, and *Carcinus maenas*, a crustacean. Successful expression using *Schwanniomyces occidentalis* glucoamylase and *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequences required considerable experimentation, and it would not be obvious to one skilled in the art that high expression of the insulin precursors would be possible using *Schwanniomyces occidentalis* glucoamylase and *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequences in view of the cited prior art.

The examiner's attention is drawn towards the fact that US Patent 5,672,487; does not mention insulin anywhere in the specification. The term protein is not exemplified to include specific disclosures. Protein is very broad term and can include anything from collagen, elastin to serum albumin, globulin, coagulation proteins, actin, tubulin, insulin, interferon, hemoglobin, enzymes, etc. For instance MF alfa signal sequence has been successfully employed for proinsulin expression in yeast, while the same fails to confer secretion of green fluorescent protein (GFP) in *S. pombe* as described above (Kjaerulff S & Jensen MR., Biochem Biophys Res Commun. 2005, 336:974-82). The disclosed protein such as hirudin cannot be equated to insulin as they are structurally and functionally class apart. There is no implicit and explicit teaching, suggestion or motivation in the either '212 or the '487 patent regarding the use of *Schwanniomyces*

*occidentalis* glucoamylase and *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequences in place of Mf-alpha signal sequences, which can lead an ordinary skill in the art to use these sequences to prepare insulin in high yield.

It is well known that in order for any prior-art references themselves to be validly combined for use in a prior-art § 103 rejection, *the references themselves* (or some other prior art) must suggest that they be combined. E.g., as was stated in *In re Sernaker*, 217 U.S.P.Q. 1, 6 (C.A.F.C. 1983):

“[P]rior art references in combination do not make an invention obvious unless something in the prior art references would suggest the advantages to be derived from combining their teachings.” That the suggestion to combine the references should not come from applicant was forcefully stated in *Orthopedic Equipment Co. v. United States*, 217 U.S.P.Q. 193, 199 (C.A.F.C. 1983):

“It is wrong to use the patent in suit [here the patent application] as a guide through the maze of prior art references, combining the right references in the right way to achieve the result of the claims in suit [here the claims pending]. Monday morning quarterbacking is quite improper when resolving the question of nonobviousness in a court of law [here the PTO].” As was further stated in *Uniroyal, Inc. v. Rudkin-Wiley Corp.*, 5 U.S.P.Q.2d 1434 (C.A.F.C. 1988), “[w]here prior-art references require selective combination by the court to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself ... *Something in the prior art must suggest the desirability and thus the obviousness of making the combination.*” [Emphasis supplied.]

In line with these decisions, the Board stated in *Ex parte Levengood*, 28 U.S.P.Q.2d 1300 (P.T.O.B.A.&I. 1993):

“In order to establish a *prima facie* case of obviousness, it is necessary for the examiner to present *evidence*, preferably in the form of some teaching, suggestion, incentive or inference in the applied prior art, or in the form of generally available knowledge, that one having ordinary skill in the art *would have been led* to combine the

relevant teachings of the, applied references in the proposed manner to arrive at the claimed invention. ...

That which is within the capabilities of one skilled in the art is not synonymous with obviousness. ... That one can *reconstruct* and/or explain the theoretical mechanism of an invention by means of logic and sound scientific reasoning does not afford the basis for an obviousness conclusion unless that logic and reasoning also supplies sufficient impetus to have led one of the ordinary skill in the art to combine the teachings of the references to make the claimed invention.... Our reviewing courts have often advised the Patent and Trademark Office that it can satisfy the burden of establishing a *prima facie* case of obviousness only by showing some objective teaching in either the prior art, or knowledge generally available to one of ordinary skill in the art, that ‘would lead’ that individual ‘to combine the relevant teachings of the references.’ ... Accordingly, an examiner cannot establish obviousness by locating references which describe various aspects of a patent applicant’s invention without also providing evidence of the motivating force which would impel one skilled in the art to do what the patent applicant has done.”

In the present case, there is no reason given in the last Office action to support the proposed combination. However the fact that both references teach DNA constructs is not sufficient to gratuitously and selectively substitute parts of one reference for a part of another reference in order to meet applicants’ novel claimed combination.

Applicants respectfully request, if the claims are again rejected upon any combination of references, that the Examiner include a explanation, in accordance with M.P.E.P. § 706.02. Ex parte Clapp, 27 U.S.P.Q. 972 (P.O.B.A. 1985), and Ex parte Levensgood, supra, a “factual basis to support his conclusion that would have been obvious” to make the combination.

It is respectfully requested that this rejection be reconsider and withdrawn.

### **Conclusion**

Applicants respectfully submit that the patent application is in condition for allowance and notification to that effect is earnestly requested. If desired, the examiner is invited to conduct a telephone conference to expedite the prosecution of the subject application. In such a case, the examiner is invited to call the undersigned attorney.

Should any official at the United States Patent and Trademark Office deem that any further action by the Applicants or Applicants' undersigned representative is desirable and/or necessary, the official is invited to telephone the undersigned at the number set forth below.

The Commissioner is hereby authorized to charge any fees which may be required regarding this application under 37 CFR §§ 1.16-1.17 or credit any overpayment, to deposit account No. 503321. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, or otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 503321.

Respectfully submitted,

By: Sam Zaghmout

O. M. (Sam) Zaghmout Ph.D  
(Registration No. 51,286)

**Contact Information:**

Bio Intellectual Property Service (BIO IPS) LLC  
8509 Kernon Ct, Lorton, VA 22079. USA

Cell Phone (703-919-4348), Fax: (703-550-0409), (703) 550-1968 (Voice/Fax)